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Studies on the Gastric Juice Protein (I)

On the "Peptide Portion" of the Gastric Juice by the Paper-Electrophoresis-Polarogram

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It was found by the polarographic protein examination that there are great quantities of dialysable polypeptide in the methanol supernatant of gastric juice.

In this paper the authors studied the protein pattern of gastric juice with P-P gram, the new method, in which polarography was taken on electrophoresed paper strips. The majority of gastric peptides moves to the cathodic side on the P-P gram, showing a quite different attitude from hitherto known gastric protein, and presents atypical protein wave with positive BPB and Pas staining.

The gastric peptides are presumably the degradation products by the enzymatic action of pepsin with hydrochloric acid in the stomach, because the peptides are present only in acidic gastric juices.

INTRODUCTION

In 1949 G. B. J. Glass and L. J. Boyd¹⁾ classified soluble mucin components of the human gastric juice into two fractions : soluble mucoprotein and soluble mucoprotease. They not only found certain clinical significances in both mucoprotein and mucoprotease, but also made an interesting observation that the isolated mucoprotein served as the intrinsic factor for Vitamin B₁₂.²⁾ On the other hand, Iwatsuru³⁾ made an early observation that there was an anemia-producing factor in the gastric juice of the patients with cancer of the stomach. The entity of this substance has been recently found to be protein.⁴⁾

The toxohormon, an extract prepared by Nakahara and Fukuoka⁵⁾ from the cancer tissues, proved also to be a kind of peptide ; a series of proteins with toxohormon-activity has been separated from the body fluids of cancer patients.

With these observations in mind, the basic study of the protein of gastric juice seems to be an important subject of research. This is particularly true in the case of the gastric juice protein from the patient with cancer of the stomach.

The present authors have been studying the properties and clinical significance of gastric juice proteins for the last several years, with special reference to the polarographic protein wave as originally described by Brdicka.⁶⁾⁷⁾

The results have been reported to the Japanese Association of Digestive

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Disease.^{8) 9) 10)} Some of the results, are as follows :

(1) The glandular mucoprotein of G. B. J. Glass shows a definite protein wave, but the mucoproteose often shows no such protein wave, except in rare cases.

Umetani¹¹⁾ reported similar results. For this reason the mucoproteose is presumably not a homogenous substance.

(2) It is interesting to note that the acetone-supernatant fluid, obtained after removing the acetone-precipitates, constitutes much higher polarographic protein wave than the above⁸⁾

Therefore, it is evident that gastric juice always contains an unknown protein fraction besides the mucoprotein and mucoproteose described by Glass. This fraction occurs more abundantly in the case of acid gastric juice than in the case of anacid gastric juice ; in any case this fraction constitutes a considerable part of the total protein wave height of natural gastric juice. Fig. 1 indicates a study where the acetone supernatant fluid from acid gastric juice, dried by evaporation at low temperature, then dissolved in distilled water again, was examined polarographically, and compared against the protein wave of the same untreated gastric juice *in toto*.

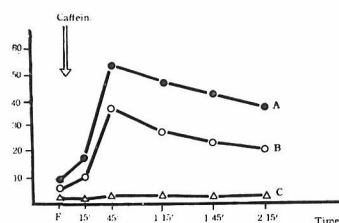


Fig. 1. Changes of protein wave-height in various proteide fractions (A, B, C) along with the process of gastric secretion (Case, No. 12).

A : Gastric juice, filtrated after addition of sulfosalicylic acid.

B : The fraction (peptide), prepared from acetone supernatant of gastric juice.

C : The fraction containing glandular mucoprotein, namely acetone precipitate of the gastric juice.

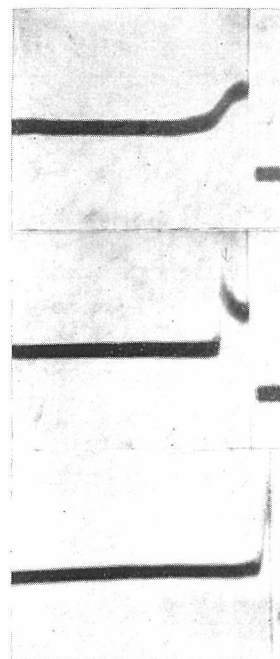


Fig. 2. Ultracentrifuge of acetone supernatant fluid. $r : 4950$; time. 1) 20min. 2) 40min. 3) 60min.

In the example shown in this case, proteid fraction from the acetone supernatant fluid is responsible for about 70% of the total wave height. This new fraction consists of protein with relatively small molecules, since they pass

through a cellophane membrane.

Studies with ultracentrifuge indicate that this fraction is a kind of peptide with such molecular weight as failing to reach an equilibrium after 50 minutes centrifugation at 49000 r. p. m. (Fig. 2). With these physico-chemical properties in mind, the authors name this fraction the gastric peptide.

Next, the peptide and mucoprotein-fraction, chemically separated, have been compared with each other in term of the wave height against concentration. The peptide fraction (Fig. 3, B) possesses a higher polarographic activity than the mucoprotein (Fig. 3, A)

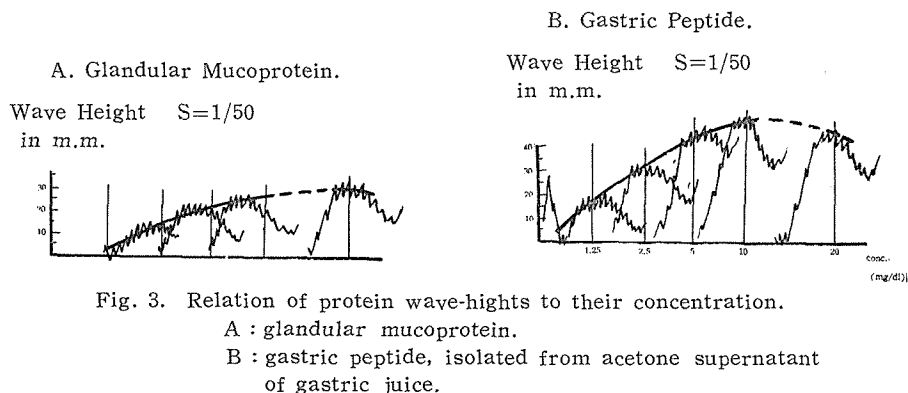
Although the polarographic activity originates in the thiol or disulfide group, this observation will not warrant an off-hand inference as to the thiol activity involved, because other factors, for instance, the protein aggregate volume,¹²⁾ can modify the situation.

Our present paper is intended to report an experiment, carried out further to assure the foregoing points, employing a combined method of polarographic protein wave study and paper-electrophoresis.

With paper-electrophoresis G. B. J. Glass, M. Rich, and L. Stephanson^{13) 14)} recently found new components named X, Y, Z, besides the hitherto known components of P, M₁, M₂... of gastric juice protein.

The present authors have been interested to know possible relations between the gastric peptide above-mentioned and the new components described by Glass.

On the other hand, we have been also interested in the relationship between the protein wave's form and the protein's nature in the case of gastric protein,



because our opinion is that the first maximum of a protein double wave originates in the protein-bound polysaccharide in general. The authors found during the course of studying serum protein denaturation that this coincides with the result of a model experiment carried out by E. De Helares¹⁵⁾.

Thus the characteristics of the protein wave's form may be studied as an important approach to the problem of the structure of protein molecule, especially in the study using the combined method of paper-electrophoresis and polarography.

Discussions on the clinical significance of the gastric juice protein will be made elsewhere.

EXPERIMENTAL PROCEDURES

(1) Gastric juice was collected with Rehfuß's gastric tube following the caffeine stimulation. Before lyophilization, this juice was filtered and neutralized with 0.1 *N* NaOH solution. A portion of the same filtered juice was lyophilized after dialysis in refrigerator for 30 hours.

(2) The paper-electrophoretic analysis of gastric juice was carried out in the same manner as by Glass, running in borate buffer at pH 9.0 and ionic strength of 0.12. Lyophilized material was dissolved. Then 0.05 ml, containing 2.5 mg. of dry material, was applied to the center of each 4 strips of filter paper (Toyo company, No. 51) 3 cm wide, and electrophoresis was carried out against the same buffer at 0.4 m.a./cm and 120 volt for 6 hours at room temperature (15–20°C).

Immediately after electrophoresis two paper strips without staining were cut each into 16 segments, 1 cm wide, and were numbered from –8 to +8– (C_8 to A_8). 8 segments on cathodic side and 8 segments on anodic side were cut out, starting from the application point. Segments of same number were put together into one jar and eluted with 2 c.c. of physiologic NaCl solution (0.9% NaCl Sol.) for 24 hours in refrigerator.

Another 2 paper strips were dried for 20 minutes at 100°C and the one was stained with BPB (bromphenol blue) stain, and the other with PAS (periodic acid Schiff) stain. All stained strips were then scanned in the densitometer.

(3) Polarography was performed on the admixture of 1 ml of this eluate and 1 ml. of Co^{+++} test solution in the thermostat at 18°C.

Sensibility of galvanometer was 1/50. The test solution used in the experiment was prepared from the following standard stock solutions prior to each examination.

- (A) 2 *N* Ammonium chloride solution.
 - (B) $2 \times 10^{-2} M$ Hexaminocobaltic chloride solution.
 - (C) 2 *N* Ammonium hydroxyde solution.
- (A) 1 vol+ (B) 1 vol + (C) 8 vols.

(added in this order)

A series of polarograms from cathodic C_5 to anode A_5 , as obtained here, was called paper-electrophoresis-polarogram of gastric juice protein. An analogous method has been applied to clinical research on normal and pathological sera by some authors, Homolka¹⁶⁾ or Kubo¹⁷⁾.

RESULTS

Paper-electrophoresis-polarograms (abbreviated as P-P gram hereafter) were run on lyophilized gastric juice substances before and after dialysis. The gastric juice used was normoacidic and obtained from normal persons. Comparing A with B in Fig. 4, it was found that the procedure of dialysis produced no change in the protein waves on anodic side of the application point (A_2 , A_3 , A_4

in Fig. 4). However on cathodic side of application point the dialysis caused disappearance ($C_4 \sim C_3$ in Fig. 4) or diminution (C_2, C_1 in Fig. 4) of some of the protein waves.

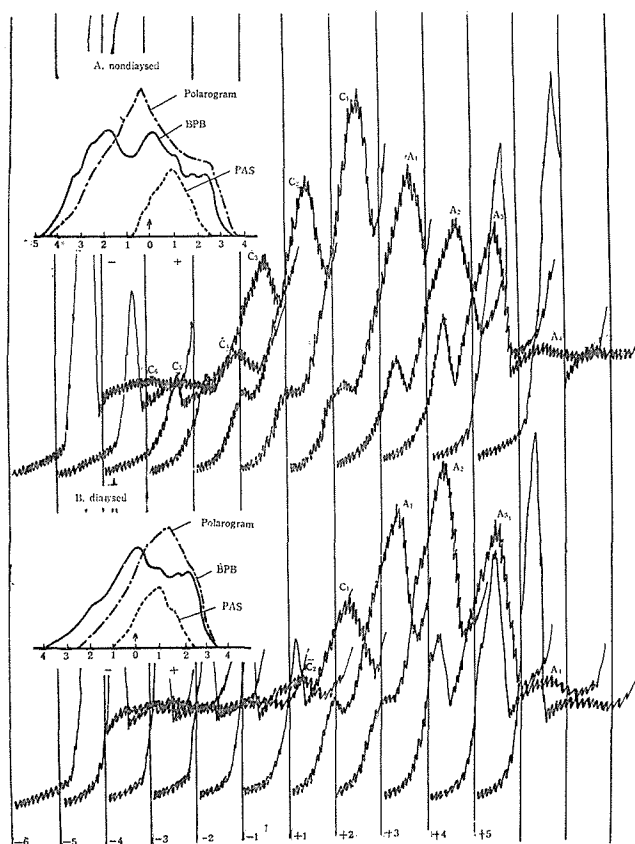


Fig. 4. Paper-electrophoresis-polarogram of nondialysed and dialysed normoacid gastric juice obtained from a normal person.

A : nondialysed.

B : dialysed.

The dialysed preparation gave more prominent cobalto-maximum than that of undialysed preparation at the extreme cathodic segment (C5).

Suspecting that the dialysable fraction may not be identical with the peptide fraction which we demonstrated in the acetone supernatant fluid of gastric juice in the previous experiment, the P—P gram of the preparation from the acetone supernatant fluid of gastric juice was also run.

As shown in Fig. 5, the peptide fraction from acetone supernatant fluid occurred always on cathodic side of the application point, either with P—P gram or in BPB stained paperelectrophoretic pattern. Therefore, it is evident that the dialysable protein fraction of gastric juice is identical with what occurs in acetone supernatant fluid.

Although the amount of peptide fraction varies from specimen to specimen

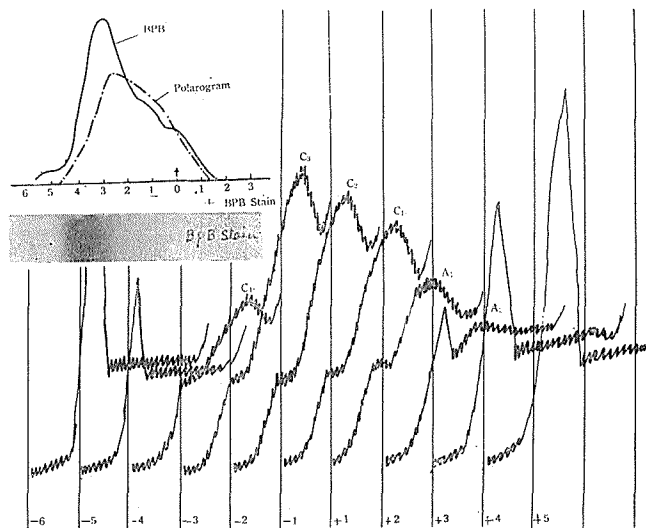


Fig. 5. Paper-electrophoresis-polarogram of the peptides in the acetone supernatant fluid.

of gastric juice, it is interesting enough that the height due to peptide depends on the presence of free hydrochloric acid in gastric juice. Generally, protein waves on cathodic side were low in cases with anacidity, but high and widely distributed over the range of C_3 , C_4 , C_5 in the cases with acidity. For instance, in Figs. 6 and 7 were presented the P—P grams obtained from two patients with cancer of the stomach, one with acid, the other with anacid gastric juice.

Common in both, wave are somewhat low on A_3 of anodic side as compared

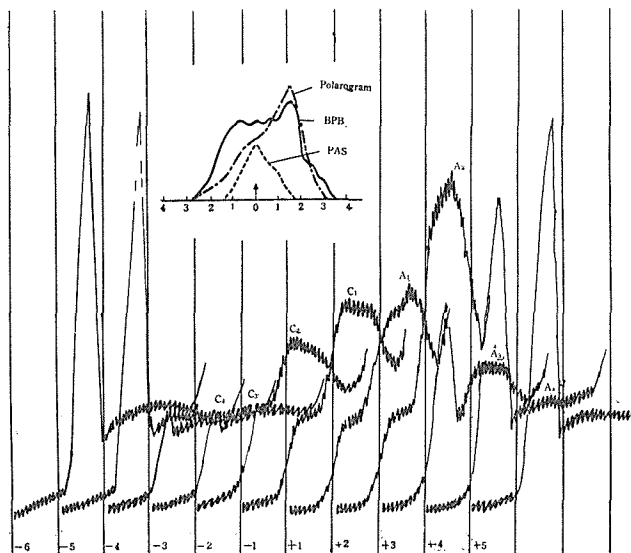


Fig. 6. Paper-electrophoresis-polarogram of anacid gastric juice obtained from a patient with gastric cancer.

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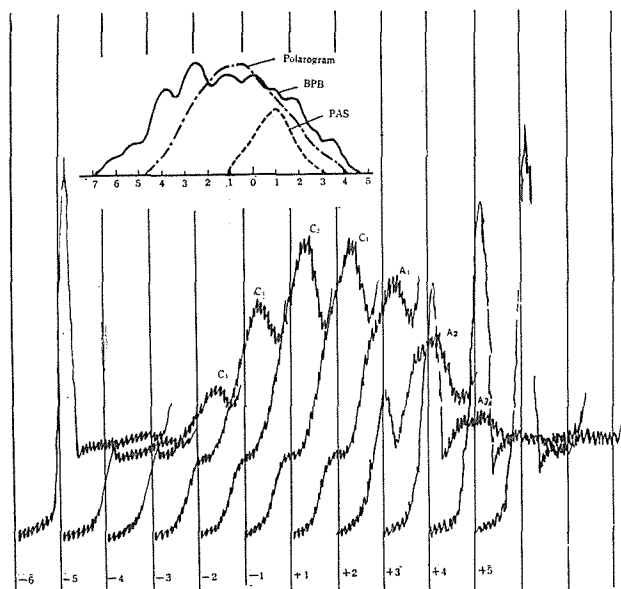


Fig. 7. Paper-electrophoresis-polarogram of acid gastric juice obtained from a patient with gastric cancer.

with normal cases, but they differ from each other in that the highest wave in the anacid case occurs on anodic side (A_2), while it occurs on cathodic side (C_1, C_2) in acid case and the latter shows wide distribution of the waves as far as to the region of C_3, C_4 , with prominent depression of cobalto-maximum at C_5 . In other words, in the case of anacid cancer protein waves are narrowly distributed both on anodic side and cathodic side of the application point, while in the case of acid cancer they are distributed narrowly on anodic side and with normal broadness on cathodic side.

Next, with regard to the wave form, the above two cancers exhibit marked difference in protein wave form at the range C_2, C_1 .

In way of introduction, some of the pertinent features of the protein wave form are as follows. A typical protein wave usually is found as a double wave (first wave and second wave). Although this double wave is variable depending upon the protein concentration, the contour of either ascending part or descending part of a wave consists of two components, thus giving the first maximum and the second maximum at -1.4, -1.6 volt respectively (Fig. 8).

The second characteristics of protein wave are a point of minimum which occurs at the point of boundary between a descending limb and subsequent ascending limb due to ammonium deposition (this occurs about at -1.8 volt and is indicated by sign M in Fig. 8). Thirdly, the degree in which cobalto-maximum wave is depressed (degree of surface activity) varies with proteins. With regard to the above mentioned characteristics of protein wave there arise certain differences between the anodic and cathodic protein waves (Fig. 10).

The anodic protein wave showed frequently typical double wave usually with deep M point. The wave at A_3 , which is presumably identified with gastric

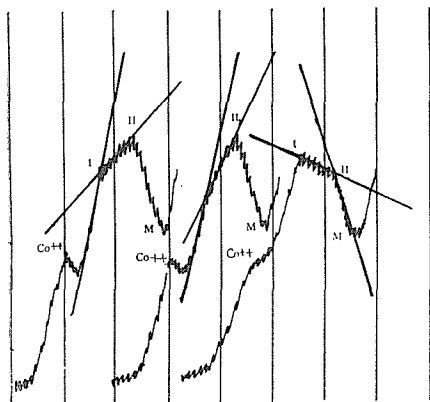


Fig. 8. Schema of protein double wave.

Co++ : cobalt-maximum.

I : first maximum.

II : second maximum.

M : minimum.

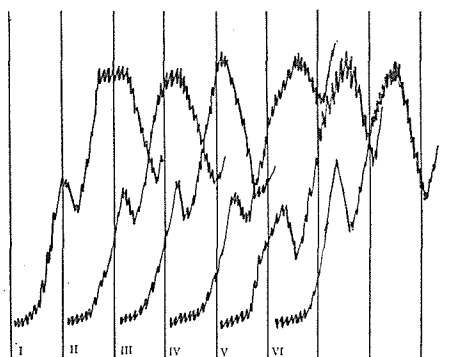


Fig. 9. Variations of the protein wave at A_3 .

I~IV : gastric juice protein.

(mucoprotein etc.)

V : serum albumin.

VI : serum albumin in an acid gastric juice.

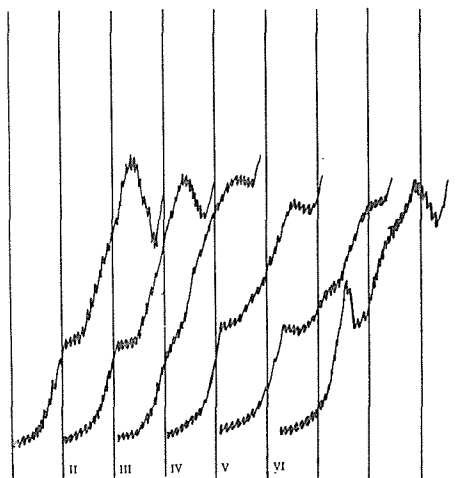


Fig. 10. Several forms of the pepitde wave at C_3 .

I~IV : gastric peptide.

V : digestion product of the blood in acid gastric juice.

VI : peptone.

mucoprotein (No. 1-No. 2 in Fig. 9), differs from that of albumin (No. 5 in Fig. 9) in shape. When blood is added to neutral gastric juice, albumin wave occurs at A_4 . With hemorrhagic gastric juice, therefore, it is possible that the undigested blood albumin may give rise to a wave at A_4 , or partially at A_3 (No. 6 in Fig. 9). In contrast, it is difficult to make out double wave on the cathodic side at $C_2 \sim C_4$ (I~IV in Fig. 10), which usually occurs as a single wave having only second maximum. This protein wave is also characterized by slightly depressed or almost absent minimum point and by marked depression of cobalto-maximum

wave. These indicate that a cathodic protein, unlike anodic protein, consists of the products of protein degradation. It is also noteworthy that even the form of peptide wave at C_3 is variable to some extent. As was mentioned before, acid gastric juice invariably gives prominent peptide wave at C_3 . When blood is added to acid gastric juice, this peptide wave at C_3 is made more prominent (V in Fig. 10). By contrast, adding blood to anacid gastric juice produces no change in protein wave at this part (C_3).

Next, relations between P-P gram and BPB or PAS stained paper-electrophoretic pattern were examined in gastric juice. With BPB there was a definite parallelism between polarographic pattern and BPB stained electrophoretic pattern (Figs. 4~7). This parallelism, however, is not a strict one, a fact accounted for by the finding that polaro-activity varies with the species of protein (Fig. 3). Further, protein wave lacked at the most cathodic end of the BPB stained areas, and cobalto-maximum was suppressed at this area (Figs. 4~7). With PAS, relations between polarogram and PAS stained paper-electrophoretic pattern was different from those with BPB, in that the cathodic side, particularly peptide fraction at C_3 does not stain with PAS at all. This indicates that the peptide does not contain polysaccharide. However, cathodic areas close to the application point (C_1 , C_2) are occasionally stained with PAS (Fig. 6). It will be recalled that these areas gave double waves polarographically, which can hardly be identified with peptide wave.

DISCUSSION

With polarographic protein analysis, we identified a new proteid fraction in the acetone supernatant of the gastric juice with peptide. This fraction moves mostly towards cathodic side during electrophoresis with borate buffer at pH 9.0. This suggests the similarity between our peptide fraction and the so-called X, Y, Z, components of G. B. J. Class.¹³⁾¹⁴⁾

Among the peptides the cathodic portion close to the application point (C_1 , C_2) persists partially even after dialysis. It gives a typical double wave form and occasional positive PAS stain. These facts suggest that this fraction is a range of transition towards the peptide and contains true protein partially. In this point we agree with Glass, who defined the X component closest to the point of application to be nondialysable.

Next, the portion more cathodic than the foregoing is dialysable. (This corresponds to Y, Z, components of Glass.)

This fraction (C_3 , C_4) gives negative PAS stain. Its wave form is characteristically for that of peptide. The acetone supernatant fluid prepared from gastric juice spread from zero to C_5 by electrophoresis, suggesting inhomogeneity of the ingredients. However, main portion as the gastric peptide occurs around C_3 , C_4 .

The tip of the stretch on cathodic side (C_5) gives neither protein wave, nor PAS staining. Here occurs, however, substances inhibitory to the cobalto-maximum, nevertheless negativity of protein wave, and at this point occurs an independent BPB spot. This suggests the existence of a low class peptide without

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SH or S-S groups.

These peptide fractions, polaro-active or not, have one common property, which give negative PAS stain. They cannot be the same substance, because even the polaro-active peptides show different wave from each other. Such a phenomenon may be partly due to the imperfect electrophoretic separations, but one cannot neglect the difference in variety of the peptides.

This peptide fraction must originate as a secondary products of digestion by pepsin within the stomach, considering experimental data on the digestion of blood in the presence of free hydrochloric acid.

However, as a matter of facts, there is a great clinical importance with respect to peptide, namely, the peptide wave becomes prominent with high frequency of incidence in the case of acid gastric of carcinomatous or precarcinomatous stomach. This dialysable peptide as well as its substrate protein must be studied throughly in the future.

As was reported previously by us, there is an interesting observation that the gastric peptide is biologically active. It causes anemia and, when given subcutaneously, produces shock-like state in experimental animals, an effect which has not been found in other protein fractions of the gastric juice.

There are many investigators in this country²⁰⁾ who found toxic substance inhibitory to liver catalase activity from tumor tissue or gastric juice from carcinomatous stomach. Such toxic substance is held partially to be peptide. In the light of these findings, it cannot be said that the peptide found by us will contain no similar cancer-specific substance.

Of anodic side protein, the mucoprotein moved as far as A₃, and showed a typical protein double wave.

A considerably high protein wave also occurred in the range of presumable mucoproteose. It is problematical whether this protein wave is solely due to mucoproteose or due to the mucoproteose admixtures with protein precipitable with sulfosalicylic acid. The eluate of this fraction gives a turbid reaction with 20% sulfosalicylic acid in the case of unacid gastric juice, suggesting the need of precise electrophoretical separation of protein from the mucoproteose.

In the previous experiment where mucoproteose has been separated chemically, occurrence of the protein wave was inconsistent, the interpretation of which, however, will require further studies.

SUMMARY

A peptide faction is present in human gastric juice particularly in acid gastric juice ; this fraction occurs in the supernatant fluid of acetone and is dialysable and gives protein waves polarographically. With combined paperelectrophoresis-polarogramm technic (P-P gram), the authors confirmed the identity between the dialysable protein fraction of gastric juice and a gastric juice component recovered in the acetone supernatant fluid. This peptide was found not homogeneous, whose main component moved to the cathodic side as far as C₃ in electrophoresis with buffer at pH. 9.0 and gave negative PAS, and positive BPB staining. This component gives a protein wave with most characteristic

wave form of peptide. It is similar to, but not identical with peptone or pepsin-digested product of the blood, and it varies with diseases.

On anodic side of P—P gram there are three to four fractions (sulfosalicylic acid precipitable protein, mucoprotein, mucoproteose). All are stainable with PAS or BPB and active polarographically with double waves and are distinguishable from admixing serum albumin on basis of wave form and mobility.

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